

Amendment under 37 C.F.R. § 1.111  
U.S. Pat. Ser. No. 10/796,166

Q80427

## **AMENDMENTS TO THE CLAIMS**

**This listing of claims will replace all prior versions and listings of claims in the application:**

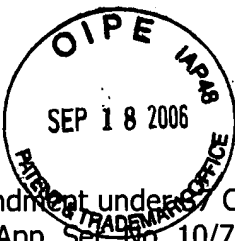
### **LISTING OF CLAIMS:**

1. **(currently amended):** A process for obtaining yeast strains conserving stress resistance in the presence of fermentable sugars, comprising the following steps  
a mutagenic treatment is carried out on cells of a starting yeast strain,  
the cells having undergone said mutagenic treatment are cultured so as to obtain a stationary phase,  
said cells in the stationary phase are incubated in the presence of at least one fermentable sugar selected from the group consisting of glucose, maltose, and sucrose, wherein said fermentable sugar is present in a quantity such that the cells enter an active metabolic state of fermentation and/or growth,  
a population of said cells in the active metabolic state is subjected to one or several stresses leading to a ~~mortality~~ survival rate of ~~at least 99%~~ equal to or lower than 1% with respect to the population,  
~~the surviving cells are isolated and~~  
yeast strains of the surviving cells that possess the following characteristics of a fil phenotype are selected;
  - growth of said selected yeast strains, evaluated by determination of at least one of the group consisting of biomass production over sugar in a given time, production yield of

biomass over sugar in a given time and growth rate, which, under identical culture conditions, is at least equal to 80% of the growth of the starting yeast strain,

- CO<sub>2</sub> release or metabolite production of said selected yeast strains, which, in identical conditions, is at least equal to 80% of the CO<sub>2</sub> release or metabolite production of the starting yeast strain,
- stress resistance of said selected yeast strains, corresponding to a survival rate at least 2 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher than the survival rate of the starting yeast strain, under identical conditions of said growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature, and
- maintenance of said growth, CO<sub>2</sub> release and stress resistance properties after repeated cultures of said selected yeast strains on non selective medium, thereby obtaining a yeast strain of the fil phenotype conserving stress resistance in the presence of fermentable sugars.

**2. (previously presented):** The process according to claim 1, wherein the selected yeast strains further present an alcohol assimilation, which, under identical conditions, is at least equal to 50% of the alcohol assimilation that of the starting yeast strain and wherein the selected yeast strains do not produce metabolites which give a bad smell or a bad or abnormal taste to breads.



**3. (previously presented):** The process according to claim 1, wherein the starting yeast strain is an industrial strain.

**4. (previously presented):** The process according to claim 3, further comprising the steps of:

- obtaining segregants from a selected yeast strain carrying several mutations,
- crossing said segregants with a laboratory haploid strain to obtain a first family of polyploids, and selecting the segregants which by crossing with the laboratory strains have produced polyploids of the first family with a glucose consumption of cells after freezing, which, under identical conditions, is equal to or higher than the glucose consumption of cells after freezing of the starting yeast strain,
- crossing of the segregants thus selected with one another to obtain a second family of polyploids,
- selection of the polyploids of the second family with a glucose consumption of cells after freezing, which under identical conditions, is higher than the glucose consumption of cells after freezing of the selected yeast strain and that possess the following characteristics of a fil phenotype:
  - growth of said polyploids of the second family, evaluated by determination of at least one of the group consisting of biomass production over sugar in a given time, production yield of biomass over sugar in a given time, and growth rate, which, under identical culture conditions, is at least equal to 80% of the growth of the starting yeast strain,

- CO<sub>2</sub> release or metabolite production of said polyploids of the second family, which in identical conditions, is at least equal to 80% of the CO<sub>2</sub> release or metabolite production of the starting yeast strain,
- stress resistance of said polyploids of the second family, corresponding to a survival rate at least 2 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher than the survival rate of the starting yeast strain, under identical conditions of said growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature, and
- maintenance of said growth, CO<sub>2</sub> release and stress resistance properties after repeated cultures of said polyploids of the second family on non selective medium,

thereby obtaining a yeast strain of the fil phenotype conserving stress resistance in the presence of fermentable sugars.

**5. (previously presented):** The process according to claim 1, wherein the yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 50% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

**6. (previously presented):** The process according to claim 1, wherein the cells obtained after mutagenic treatment are introduced into pieces of dough subjected to at least 100 cycles of freezing/thawing after a first fermentation of the dough of 30 minutes at 30°C.

**7. (previously presented):** An industrial isolated yeast strain of the fil phenotype obtainable by the process according to claim 1.

**8. (canceled).**

**9. (previously presented):** The industrial isolated yeast strain according to claim 7, belonging to *Saccharomyces cerevisia* species.

**10. (previously presented):** The industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on glucose of 10 minutes at 30°C after stationary phase.

**11. (canceled).**

**12. (previously presented):** The industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and

160 mg of dry matter of the considered strain, defined by the ratio between the release of CO<sub>2</sub> at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO<sub>2</sub> at 30°C after 1 day of conservation at -20°C, is at least equal to 80%.

**13. (canceled).**

**14. (previously presented):** The industrial isolated yeast strain according to claim 57, presenting a decrease in gassing power after drying of biomass of said industrial isolated yeast strain harvested in a phase close to exponential growth phase, which is at most equal to 67% of the decrease in gassing power of the starting yeast strain under identical conditions.

**15. (previously presented):** A strain PVD1150 = M5 *f11* deposited at Collection Nationale de Cultures Microorganismes (C.N.C.M.) under the n° I-2031 and the n° I-2203.

**16. (previously presented):** A strain KLI = W303 *f12* deposited at C.N.C.M. under the n° I-2032.

**17. (previously presented):** A strain FD51 = HL816 *f1300* deposited at C.N.C.M. under the n° I-2033.

**18. (previously presented):** A strain FDH16-22 = HL822 *f1300* deposited at C.N.C.M. under the n° I-2034.

**19. (previously presented):** A strain AT25 = *S47 fl400* deposited at C.N.C.M. under the n° I-2035.

**20. (previously presented):** A strain AT28 = *S47 fl1500* deposited at C.N.C.M. under the n° I-2036.

**21. (previously presented):** A strain AT251 deposited at C.N.C.M. under the n° I-2222.

**22. (previously presented):** A strain AT252 deposited at C.N.C.M. under the n° I-2223.

**23. (previously presented):** A strain AT254 deposited at C.N.C.M. under the n° I-2224.

**24.–37. (canceled).**

**38. (previously presented):** A dry baker's yeast obtained by culturing an industrial isolated yeast strain according to claim 7.

**39. (canceled).**

**40. (previously presented):** A brewery yeast obtained by culturing an industrial isolated yeast strain according to claim 7.

**41. (previously presented):** A yeast intended for the production of alcohol obtained by culturing an industrial isolated yeast strain according to claim 7.

**42. (previously presented):** The process according to claim 1, wherein the yeast strains are of the *Saccharomyces cerevisiae* species.

**43. (previously presented):** The process according to claim 1, wherein the selected yeast strains present growth of said selected yeast strains, evaluated by determination of at least one of the group consisting of biomass production over sugar in a given time, production yield of biomass over sugar in a given time, and a growth rate, which, under identical culture conditions, is at least equal to 90% of the growth of the starting strain.

**44. (previously presented):** The process according to claim 1, wherein the selected yeast strains present CO<sub>2</sub> release or a metabolite production of said selected yeast strains, which, in identical conditions, is at least equal to 90% of the starting yeast strain.

**45. (previously presented):** The process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 3 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 2 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower



temperature.

**46. (previously presented):** The process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 5 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 3 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower temperature.

**47. (previously presented):** The process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 10 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 5 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower temperature.

**48. (previously presented):** The process according to claim 1, wherein the obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 60% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

**49. (previously presented):** The process according to claim 1, wherein the obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 70% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

**50. (previously presented):** The process according to claim 1, wherein the obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 80% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

**51. (currently amended):** An industrial isolated yeast strain according to claim 7 belonging to the *Saccharomyces* genus.

**52. (previously presented):** An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 60% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

**53. (previously presented):** An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 70% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of

10 minutes at 30°C after stationary phase.

**54. (previously presented):** An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 75% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

**55. (previously presented):** An industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour 15 of water 1 g of sucrose, 0.405 of NaCl, 0.06 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 160 mg of dry matter of the considered strain, defined by the ratio between the release of CO<sub>2</sub> at 30°C after 1 month or 30 days of conservation at -20°C, and the release of CO<sub>2</sub> at 30°C after 1 day of conservation at -20°C, is at least equal to 85%.

**56. (previously presented):** An industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 160 mg of dry matter of the considered strain, defined by the ratio between the release of CO<sub>2</sub> at 30 °C after 1 month or 30 days of conservation at -20°C and the release of CO<sub>2</sub> after 1 month or 30 days of conservation at -20°C and the release of CO<sub>2</sub> at 30°C after 1 day of conservation at -20°C, is at least equal to 90%.

**57. (previously presented):** An industrial isolated yeast strain of the fil phenotype, obtainable by the process according to claim 7, presenting an alcohol assimilation, which, under identical conditions, is at least equal to 50% of that of the starting yeast strain and not producing metabolites which give a bad smell or a bad or abnormal taste to breads.

**58. (previously presented):** An industrial isolated yeast strain according to claim 57, presenting a decrease in gassing power, after drying of biomass harvested in a phase close to exponential growth phase, which, is at most equal to 50% of the decrease in gassing power of the starting yeast strain under identical conditions.

**59. (previously presented):** A baker's yeast obtained by culturing an industrial isolated yeast strain according to claim 7.

**60. (previously presented):** An industrial isolated yeast strain of the fil phenotype obtainable by the process according to claim 1 and, presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06g of  $(\text{NH}_4)_2\text{SO}_4$  and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 60%, said stability being defined as the ratio between the release of  $\text{CO}_2$  at 30°C after 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

**61. (previously presented):** An industrial isolated yeast strain of the fil phenotype

obtainable by the process according to claim 1 and, presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of  $(\text{NH}_4)_2\text{SO}_4$  and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 80%, said stability being defined as the ratio between the release of  $\text{CO}_2$  at 30°C after 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

**62. (previously presented):** An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing of fermentable sugar of 10 minutes at 30°C after stationary phase.